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TCR $\alpha\beta^+$ and CD19 $^+$ cell depleted Haploidentical and Mismatched Hematopoietic Stem Cell Transplantation in Primary Immune Deficiency

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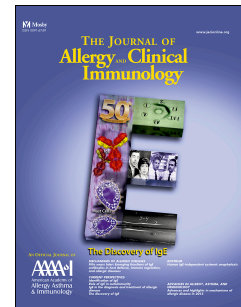
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Title Page

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TCR $\alpha\beta^+$ and CD19 $^+$ cell depleted Haploidentical and Mismatched Hematopoietic Stem Cell Transplantation in Primary Immune Deficiency

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Abstract:

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is used as a therapeutic approach for primary immunodeficiencies (PIDs). The best outcomes have been achieved using HLA-matched donors, but when a matched donor is not available, a haploidentical or mismatched unrelated donor (mMUD) can be useful. Various strategies are used to mitigate the risk of graft versus host disease (GvHD) and rejection associated with such transplants.

Objective: We sought to evaluate the outcomes of haploidentical or mMUD HSCT after depleting GvHD causing TCR $\alpha\beta$ CD3⁺ cells from the graft.

Methods: CD3⁺TCR $\alpha\beta$ ⁺/CD19⁺ depleted grafts were given in conditioned (except three) children with PIDs. Treosulfan (busulfan in one), fludarabine, thiotepa and ATG or alemtuzumab conditioning was used in 77% cases, and all but four received GvHD prophylaxis.

Results: 25 patients with 12 types of PIDs received 26 HSCTs. Three were transplanted for refractory GvHD developing after first cord transplantation. At 20.8 months (5 month- 3.3 years) median follow up, 21/25 patients survived and were cured of underlying immunodeficiency. Overall and Event Free Survival at 3 years was 83.9% and 80.4% respectively. Cumulative incidence (CI) of Grade II-IV acute GvHD was 22 $\pm$ 8.7%. No case of visceral or chronic GvHD was seen. CI of graft failure, CMV or/and adeno viral infections and transplant related mortality at 1 year were 4.2 $\pm$ 4.1%, 58.8 $\pm$ 9.8% and 16.1 $\pm$ 7.4% respectively. Patients going into transplant with systemic viral infections had poor survival in comparison to those with absent or resolved infection (33.3% vs.100%).

Conclusion: CD3⁺TCR $\alpha\beta$ ⁺ and CD19⁺ depleted haploidentical or mMUD HSCT is a practical and viable alternative for children with a range of PIDs.

Clinical Implications:

This paper supports the use of CD3+TCR $\alpha\beta$ ⁺ and CD19⁺ depleted haploidentical or mMUD HSCT across a range of PIDs and should be considered in children with PID lacking an HLA matched donor or when urgent HSCT is indicated.

Capsule Summary:

CD3+TCR $\alpha\beta$ ⁺ and CD19⁺ cell depleted haploidentical or mMUD HSCT has reproducible outcomes in children with a range of PIDs resulting in good immune reconstitution. Viral infections before or after HSCT are major contributors to mortality and morbidity.

Key words:

Primary immunodeficiency, Haploidentical, mismatched unrelated, hematopoietic stem cell transplantation,
CD3+TCR $\alpha\beta$ ⁺ cell depletion

Abbreviations:

ADV: Adenovirus

ATG: Anti-Thymocyte Globulin

BCG: Bacillus Calmette–Guérin

CI: Cumulative Incidence

CID: Combined Immune Deficiency

CMV: Cytomegalovirus

CsA: Cyclosporine

CTLs: Cytotoxic T Lymphocytes

EBV: Epstein - Barr virus

EFS: Event Free Survival

G-CSF: Granulocyte colony stimulating factor

GvHD: Graft-versus-host disease

HHV6: Human herpes virus 6

HLA Human Leukocyte antigen

HLH: Hemophagocytic Lymphohistiocytosis

HSCT: Hematopoietic stem cell transplantation

IRIS: Immune Reconstitution Inflammatory Syndrome

mMUD: mismatched unrelated donor

NK: Natural Killer

OS: Overall Survival

PID: Primary immunodeficiency

SCID: Severe combined immunodeficiency

TCD: T-cell depletion

TMA: Thrombotic Microangiopathy

TPN: Total Parenteral Nutrition

WAS: Wiskott Aldrich Syndrome

Introduction:

HSCT is curative for many primary immunodeficiencies (PIDs); however an HLA-matched donor may not be available and in this case, mismatch related (haploidentical) or unrelated donor (mMUD) can be useful. Due to significant HLA donor-recipient disparities, these options bring higher rates of graft failure and GvHD which contribute to poor survival.¹ Various approaches have been tried to mitigate these risks; for example, T-cell depletion², T and B-cell depletion³, mega dose CD34+ cell selection⁴, and recently post-transplant cyclophosphamide⁵, CD3+TCR $\alpha\beta$ ^{6, 7} and naïve T-cell depletion.⁸ Commonly used methods such as CD34+ selection decrease the risk of GvHD but the risk of delayed viral clearance and immune recovery remains.⁹ Graft manipulation by depleting GvHD-inducing α/β receptor T-cells offers a reduction in GvHD while retaining populations of γ/δ receptor T-cells¹⁰ in the graft, which may contribute to early immune reconstitution and viral or tumor clearance^{11, 12} after HSCT. Recent studies have shown beneficial effects of this approach in both malignant¹²⁻¹⁴ and non-malignant disorders.¹⁵⁻¹⁷ Using this technique to transplant from an immediately available haploidentical donor seems appealing, but more data are required not only on survival but also on morbidities, long-term immune reconstitution and cost effectiveness in comparison to other modalities of transplant. We report the outcome of this method in 25 patients with PIDs undergoing mismatched HSCT when a suitably matched donor was not available.

Methods:

A retrospective review of consecutive patient records was performed. Pre-transplant data were collected regarding: patient demographics, infections, donor-recipient matching, conditioning regimen. Post-transplantation data were collected on engraftment, transfusion requirement, immune reconstitution, GvHD, and infections. Chimerism analysis was performed by PCR-based amplification of STR sequences. Various cell types in blood were assessed by flow cytometry using monoclonal antibodies against lineage-specific surface molecules (T-cells- CD3+, NK cells- CD16+/CD56+, B-cells- CD19+, Naïve T-cells- CD45RA+/CD27+). In addition, morbidities (pain, organ toxicities, and nutritional support), the length of hospitalization and long-term complications were recorded. All parents gave consent according to EBMT and local center regulations. Information regarding patients 1, 7 and 20 has been previously published.¹⁸⁻²⁰

Patients

Patients' demographics and transplantation characteristics are shown [Table I]. 25 patients underwent 26 transplants at the two HSCT centers for PIDs in the UK [Great North Children's Hospital, Newcastle (n=18), and Great Ormond Street Hospital, London (n=8)] between October 2012 and September 2016. The median age at transplant was 1.75 years (range, 0.28-10.3). Infants (≤ 1 year) comprised 34.6% of the cohort. Two patients received a graft from mMUDs; one with an A mismatch and another with DR and DQ mismatches. The median referral-transplant interval was 4 months (0.5-16) (n=21). Three patients underwent a second conditioned transplantation for refractory GvHD following matched (10/10) cord blood HSCT. Regarding their first HSCT, 2/3 were conditioned. All three had skin GvHD while patient 8 also had GvHD affecting the joints and bone marrow resulting in aplasia and patient 9 had grade III liver and gut GvHD (biopsy proven). One patient received CD3+TCR $\alpha\beta$ ⁺/CD19⁺ depleted HSCT twice due to primary graft failure after the first transplant. All patients underwent blood, bronchoalveolar lavage (Newcastle only), nasopharyngeal aspirate, stool and urine screening for infections within 10 days pre-transplant.

Transplantation characteristics

The source of stem cells was G-CSF mobilized peripheral blood in all patients. All grafts were TCR $\alpha\beta^+$ /CD19 $^+$ depleted using an immunomagnetic method following the manufacturer's recommendations (Miltenyi Biotec, Bergisch Gladbach, Germany). Most patients (80.7%) received reduced-toxicity myeloablative conditioning consisting of treosulfan (36 to 42 g/m ^{2} total dose; days -7 to -5) [busulfan in one], fludarabine (160 mg/m ^{2} total dose; day -7 to -4), and thiotepea (10mg/kg; day -4). Three SCID patients did not receive any conditioning (one each due to disseminated BCG, prematurity and disseminated CMV). All conditioned patients except one (with drug-resistant CMV) received serotherapy [ATG-Fresenius®(rabbit) n=18, Alemtuzumab n=4]. ATG (15 mg/kg total) was given over days -5 to -3, and alemtuzumab (1mg/kg total) was given over days -8 to -4. Six patients received 200 mg/m ^{2} rituximab (in addition to ATG) on D-1 to reduce the risk of EBV-related post-transplant lymphoproliferative disease. Post-transplant GvHD prophylaxis was given in 22/26 transplants [Table I]. CsA (trough target 50-100 μ mol/L) and MMF were used for 12 and 6 weeks post-HSCT, respectively, unless required longer for GvHD. Steroid (1mg/kg/day) was added for GvHD prophylaxis in patients with refractory GvHD and Omenn syndrome for around 2 weeks post HSCT and then tapered off over the next 4-6 weeks. GvHD was graded according to the modified Seattle Glucksberg criteria²¹. All patients received ursodeoxycholic acid, and five patients received defibrotide as veno-occlusive disease (VOD) prophylaxis (one patient with transaminitis receiving busulfan conditioning, one with liver involvement due to HLH, one with grade III GvHD affecting the liver and two with abnormal liver function tests). All patients received immunoglobulin replacement post-HSCT until sustained IgM production was demonstrated. Viral infections were treated pre-emptively. CMV, EBV, adenovirus, (and HHV-6 only in Newcastle) were monitored weekly in the blood by quantitative PCR. Respiratory (nasopharyngeal aspirate) and gut (feces) viral screens were performed weekly.

Statistical Analysis

Data were analyzed as of 20th February 2017 with STATA version 14.1. Patients were censored at the time of death or last follow-up. The probabilities of overall and event-free survival were estimated by the Kaplan-Meier product limit method. For calculation of EFS, death, disease persistence and graft failure were considered as events. Primary graft failure was defined as the neutrophil count never reaching $\geq 0.5 \times 10^9$ /L. The probabilities of GVHD, graft failure, viral infections, and transplant-related mortality were calculated as cumulative incidence curves $\pm$ standard error to adjust the analysis for competing risks. Multivariate Cox regression model examining risk factors for GvHD were built with the use of stepwise forward selection, with the P value of 0.05 or less considered to indicate statistical significance. Variables considered were, use of different serotherapy in conditioning, viral reactivation status, doses of various cells in the graft.

Results:

Data on different cell population in the processed grafts are given in Table II. The median proportion of remaining TCR $\alpha\beta$ T-cells in the product after depletion was 0.28% (range: 0.08-9.7). No patients experienced adverse reactions to the infusion of stem cell product.

Neutrophil count recovery occurred in all but two conditioned patients with a median of 15 days (range: 10-27). One patient developed primary graft failure, and another died on D+13. The cause of graft failure could not be determined as virology testing was negative and anti-HLA antibodies were present at a low level but were unreactive with patient's cells. This patient was successfully re-transplanted using CD3+TCR $\alpha\beta$ ⁺/CD19⁺ depleted stem cells from an mMUD donor. None of the patients required G-CSF to achieve engraftment or had secondary graft failure. Patient 8 [Table I] was given buffy coat granulocyte infusions in the peri-transplant period because of pre-existing fungal infection. The median time to platelet engraftment was 9.5 days (range: 0-24), defined as platelet count $>20 \times 10^9/L$ for 7 days ($>50 \times 10^9/L$ for WAS patients). Acute GvHD developed in 11/23 patients [Table II] (three cases are excluded: one with graft failure and two with early death); however, 10/11 had only Gr I/ II skin GvHD and responded to first line treatment. 2/10 patients had a recurrence of skin GvHD after CsA discontinuation, and both responded to a short course of steroids. On multivariate analysis, GvHD was not associated with the dose of CD34⁺, T cells, TCR $\alpha\beta$ ⁺, CD19⁺ and NK cells in the graft or the different serotherapy in conditioning, systemic adenovirus or CMV infections.

As of February 2017, 21/25 patients are alive, and disease free, with a median follow-up of 20.8 months. The probability of OS and EFS at 3 years for the whole cohort was $83.9 \pm 7.4\%$ [95% CI 69.3-98.4] and $80.4 \pm 7.9\%$ [95% CI 64.9-95.8], respectively. As of the last follow-up, 76.1% survivors had full donor chimerism and 5 patients had high T-cell but mixed myeloid chimerism (2 unconditioned) [Table I].

Transplant related mortality (TRM) and morbidities

TRM at 100 days was 11.7% [95% CI 4.0-33.9] and at one year was 16.1% [95% CI 6.5-39.7] with four deaths. Patient 5 with drug-resistant CMV did not receive serotherapy during conditioning, and developed grade III skin GvHD requiring prolonged immune suppression but died from aspergillosis. Two infants (no. 10, 21) with disseminated CMV before transplant died of pulmonary complications soon after transplant. Patient 12 died two months after transplant due to pulmonary hemorrhage and pulmonary hypertension in the presence of ongoing adenoviremia. Patients going into transplant with active CMV or adenovirus infection had significantly worse survival in comparison to those with no active viral infections [33.3% vs. 100%, $p < 0.0001$] [Fig. 1].

Early conditioning related toxicities, such as treosulfan-related skin rash, mucositis, and engraftment syndrome, were mild in most cases. Patient 9 with pre-existing skin GvHD developed a severe treosulfan rash which required dressings under sedation. Patients 7, 14, 15 and 21 developed severe mucositis. Among conditioned patients, 56.5% required systemic analgesic infusion for at least 72 hours. Patients 7 and 12 developed capillary leak syndrome around engraftment and needed oxygen support and diuretics. The most significant non-infectious complications were immune reconstitution inflammatory syndrome (IRIS) ($n=2$) and TMA ($n=2$). Patient 5 with disseminated BCG before transplant and patient 20 with disseminated enterovirus infection after transplant developed IRIS²⁰. TMA developed in patients 7 and 26 which responded to supportive measures and defibrotide¹⁹ in one and eculizumab²² in another. Three patients developed features of posterior reversible encephalopathy syndrome, which responded to discontinuation of CsA. None of the patients developed VOD. The median number of blood and platelet transfusions after transplant was 4 (0-29) and 2 (0-46) respectively. Patients with WAS became platelet transfusion independent within 1 week after transplant. The median D0-discharge interval in survivors was 86 days (34-327).

Viral infections or reactivations post-transplant

Twelve patients had viral infections requiring antiviral therapy within 10 days pre-transplant, and 50% were able to clear their virus by day 0. Six children went into transplant with systemic viremia (CMV=4,

Adenovirus=2) of which four died. Of these, the blood viral PCR titre fell to $<2 \times 10^3$ copies/ml by day 0 in three patients with antiviral drugs (one died, two surviving) and blood viral PCR remained at a higher level $>10 \times 10^3$ /ml in the remaining patients and all three died. 18 patients had 26 systemic viral infections or reactivations post-transplant (CMV=10, Adeno=9, HHV-6=5, EBV=1, enterovirus=1). Among these, six infection episodes (33.3%) (HHV-6=3, Adeno=2, EBV=1) in three patients did not result in any complications nor require treatment as the titres fell with immune recovery. The CI of any significant viral infection post-transplant (rising blood titres, disseminated or symptomatic infection) was $59.9 \% \pm 11.1\%$ [Table II]. The median time to clear the virus from blood was 75.5 days (18-210). Patient 3,4 and 14 received CMV-specific CTLs on D+34,D+31 and D+7 respectively at a dose of 1×10^4 CD3 cells/kg since their viral titres remained $>100,000$ copies/ml despite antiviral treatment. Patient 19 received adeno-specific CTLs on D+47 as a part of ASPIRE (ADV-specific T-cells in Paediatric patients post allo-HSCT) trial. Except patient 14, none of these patients had evidence of CMV or adenoviral disease at the time of CTL infusion; however, patients 3 and 4 developed CMV retinitis around 2-3 weeks after CTL infusion and were managed with intraocular foscarnet along with systemic antivirals. Five patients (3 with CMV, 2 with Adenovirus) did not clear viruses post-transplant despite treatment, and 4/5 died. To date, no patient has developed EBV-related post-transplant lymphoproliferative disease.

Twelve patients went into transplant with enteric viral infections (Norovirus=7, Adenovirus=5, vaccine-associated Rotavirus=3). Five additional patients were found to have viral infections post-transplant (Norovirus=4, Enterovirus=1, Sapovirus=1). Most of these patients had feeding intolerance and poor weight gain. Out of 11 patients with Norovirus infection, nine could clear it at a median of 243 days (range: 122-590) post-transplant. All patients showed symptomatic improvement before viral clearance from the gut. Patients were managed with enteral feeding (oral or nasogastric tube) or TPN-as required. 80.7% of patients required TPN at some point during the post-transplant period. Out of these 7 (33.3%) patients required TPN for >80 days post-transplant and all had gut viral infections (Norovirus=4, Rotavirus=2, Norovirus+ Enterovirus=1).

Immune reconstitution

Co-transfused NK cells were detectable in the first week post-transplant, and the NK cell counts remained >200 cells/microL in most patients after D30. Median time to CD4 recovery (absolute count ≥ 200 on 2 consecutive tests) was 129 days (79-515) in 20 patients (three were early deaths and data for one patient is not available). Naïve T-cells appeared at four months post-transplant in most patients. Median time to B-cell recovery (count ≥ 200 cells/microL on two consecutive tests) in all conditioned but two patients (n=17) was 85 days (range 41-367). The B-cell count of patient 13 remained zero until his death nine months after HSCT despite good T-cell recovery and 100% donor chimerism. Class-switched memory B cell (CD27+IgD-ve) testing at 6-month post-transplant showed median of 1% (0-9) subpopulation of these cells in 14 patients and the figures at 1 year and 1.5 year were 2% (1-4) and 3% (1-5), respectively. Considering robust absolute B-cell numbers at these time points (figure 4), this proportion of class-switched memory B-cells heralds good immune recovery. The median days to achieve normal IgM level (>0.5 g/L on two separate occasions) in 15 survivors was 97 days (62-187). Only 5 surviving patients have IgM level <0.5 g/L two of them are <6 months post-transplant. Flow cytometric analyses performed on peripheral blood samples 4 weeks after HSCT showed that $\gamma\delta$ T-cells represented the majority of T-cells (mean 81.5%, range: 32-100). Subsequently, the $\alpha\beta$ T-cell proportion gradually increased. A normal $\alpha\beta/\gamma\delta$ ratio was reached around one year after transplantation.

Long-term follow-up and complications

All surviving patients are doing well. Four patients developed autoimmune cytopenias 4-17 months post-transplant and responded to steroids or/and immunoglobulins. One patient has mild chronic renal insufficiency and is on anti-hypertensive therapy. One patient developed prolonged (8 months) renal TMA which has now resolved. Of available data on 17 patients surviving more than one year post-transplant, 14 stopped requiring Ig replacement (indirect evidence of class switched memory B cell function) and commenced primary immunizations.

Discussion:

We report a 3 year EFS of 80.4% after CD3+TCR $\alpha\beta$ ⁺/CD19⁺ depleted HSCT in the most diverse group of PID patients reported to date; some of whom historically had poor outcomes using haploidentical donors.¹ Two studies^{15, 16} have been published using the same approach for PIDs [Table II] and the major difference in comparison to our study is the rate of graft failures. Balashov et al¹⁵ showed a high frequency of graft failure which may be related to the use of only one alkylating agent for conditioning in 9/10 patients with graft failure. In the study by Bertaina et al.¹⁶, 17.4% patients developed graft failure despite getting two alkylating agents, but all were in diseases known to be associated with an increased risk of rejection (thalassemia, osteopetrosis, and severe aplastic anemia). Use of an additional alkylating agent may have helped clear host stem cell niches as evident by only one case of graft failure in our study. The addition of a second alkylating agent did not seem to result in additional short-term toxicities in both previous studies and the current study where about half of conditioned patients did not even require systemic analgesia.

Despite using GvHD prophylaxis in our cohort, 47.8% of patients developed aGvHD (mild in 10/11). It is important to note that 5/11 patients developed skin GvHD around the time of engraftment (range: D+13 to D+17). We did not perform skin biopsies and have a lower threshold of reporting them as skin GvHD when many centers would pass them as engraftment syndrome. In the Italian study, only 3/23 patients developed mild GvHD despite no use of GvHD prophylaxis. Rituximab has been shown to reduce the incidence of acute GvHD²³ and rituximab was used in all of their patients; however, both B-cell dose in the graft and use of rituximab was not found to be associated with GvHD on multivariate analysis in our study. But the number of patients receiving rituximab is small (n=6) to derive any definite conclusion. Interestingly, a median dose of B-cells in the grafts in Italian study was a log lower compared to our study, although the reason for this is not clear. Although we were able to restrict the TCR $\alpha\beta$ cell dose to $<1 \times 10^5$ /kg, it is possible that remaining TCR $\alpha\beta$

or TCR $\gamma\delta$ cells in the graft might have played a role in the occurrence of aGvHD. Whilst $\alpha\beta$ T-cell subsets are known effectors of GvHD, the role of $\gamma\delta$ T-cells in GvHD is unclear with conflicting results from animal and human studies with some showing a beneficial role in reducing GvHD^{24, 25} while others suggest pro-GvHD effects.²⁶⁻²⁸ A recent study²⁹ showed a lower frequency of naïve $\gamma\delta$ T-cells in the peripheral blood of donors corresponding to patients who later developed grade II–IV aGVHD.

Interestingly, we saw only skin GvHD in the current study as in the Italian cohort.¹⁶ A similar trend was noticed in a study in leukemia patients by Maschan et al¹³: 40% CI of isolated skin GvHD in TCR $\alpha\beta$ depleted group versus 6% in matched unrelated transplant group, although this was not statistically significant. TCR $\gamma\delta$ cells are found in high concentration in skin epithelia and gut mucosa under physiological conditions and it is possible that TCR $\gamma\delta$ T-cells might have ‘homed’ into the skin or gut after transplantation but this doesn’t explain the absence of gut GvHD in our cohort. On the contrary, it is likely that small amount of TCR $\alpha\beta$ cells in the graft were able to induce mild skin GvHD but did not result in any systemic GvHD. This finding needs further investigation as we have not done subset analysis of infiltrating T-cells in GvHD lesions. Moreover, three patients underwent transplant for refractory GvHD and considering their underlying inflammatory milieu they had a very high risk of significant GvHD and still, none of them developed visceral or severe skin GvHD. This suggests TCR $\gamma\delta$ cells are less likely to cause GvHD and TCR $\alpha\beta$ depletion is a good method to prevent GvHD. With the possible effect of GvHD on the thymus and other detrimental effects on the quality of life, any GvHD is undesirable in a patient with the non-malignant disease. Thymic damage due to GVHD³⁰ could result in mature medullary epithelial cell depletion and a defect in negative selection³¹ which may lead to a higher risk of chronic GvHD³⁰ and possibly autoimmune illnesses. We, therefore, favored using low dose CsA. Although we have not seen cGvHD in our cohort, four patients developed autoimmune cytopenias post-transplant, 3 of whom had aGvHD.

It is clear from the evidence that TCD by negative selection (T-cell depletion of the graft) is a better method to reduce the risk of GvHD compared to positive CD34 selection but it is associated with an increased risk of graft failure. It is difficult to compare the current study with studies using different ex-vivo TCD strategies for HSCT in children with PIDs as our cohort comprises patients with diverse non-SCID PIDs (which

have a higher risk of graft failure vs. SCID population) compared to the majority of SCID patients in previous studies³²⁻³⁴ [Table S1 in supplement]. However, based on our data and previous reports using TCR $\alpha\beta$ depletion, it is clear that graft failure rate is low with this technique and rapid engraftment is achieved in most patients. We suspect that apart from the conditioning, residual TCR $\gamma\delta$ cells, and other immune cells contribute to robust engraftment and a very low rate of graft failure. It has been shown that infusion of activated $\gamma\delta$ T-cells, can promote engraftment^{35, 36} without causing GvHD even in the MHC-mismatched situation.³⁷

Patients going into transplant with active viral infections had the worst outcomes in our cohort as shown previously in SCID patients undergoing HSCT where infants (>3.5 months of age) with infection had a poor survival in comparison to those with resolved infections or those without prior infection (50% vs. 82% vs. 90%).³³ One of the deaths in our cohort was at 9 months post-transplant due to aspergillosis resulting from prolonged immune suppression for GvHD. This patient had persistent low-grade CMV viremia at the time of death which is well known to contribute to both immune suppression^{38, 39} and heightened the risk of GvHD⁴⁰ requiring immunosuppression-both risk factors for fungal infections. Viral infections in HSCT are known to have unfavorable consequences and increased cost with longer hospital inpatient days.⁴¹ We do not think the use of low dose CsA in our cohort contributed to the occurrence of viral infections which is comparable to the Bertaina et al¹⁶. data (no use of CsA) and to the published frequency of viral infections (51.6%) in pediatric patients with non-malignant illnesses post MSD or MUD transplant using reduced intensity conditioning with regular dose GvHD prophylaxis.⁴² Extensive weekly virology screening including HHV-6 and Enterovirus might have picked up a higher number of asymptomatic infections in our cohort. An active role of $\gamma\delta$ T-cells in the immune response against CMV^{43, 44} has been suggested. Whilst there may be some beneficial anti-CMV effect from infused $\gamma\delta$ T-cells (as shown by us previously¹⁸ in patient 1) not all patients were able to clear CMV. Out of 6 patients with disseminated CMV or adeno pre-transplant, only two survived HSCT despite the use of antivirals. A recent study⁴⁵ showed that, although TCR $\alpha\beta$ depletion strategy is associated with high CMV reactivation rates, it does not affect the outcomes of such transplants.

Similar to previous studies³⁴, we have shown robust immune reconstitution 4 months after HSCT. Additionally, patients came off CsA at a median of 4 months (range 2.5-9) post-HSCT and a majority in longer follow up are

off immunoglobulins [Table S1 in supplement]. Assuming most children will have a haploidentical donor available, use of this technique will result in a shorter diagnosis to transplant interval which in turn can lead to fewer pre-transplant infections which are known to contribute to mortality and morbidity. Availability of donor cells for CTL therapy at a later date will be an additional advantage⁴⁶. The cost of the consumables per CD3+TCR $\alpha\beta$ ⁺/CD19⁺ depletion procedure (including apheresis cost) is around £10000 which is cheaper than procuring a MUD (£15-20000) or UCB (£17-35000) donor.

We conclude, the use of CD3+TCR $\alpha\beta$ ⁺ and CD19⁺ depletion for ex vivo processing of mismatched donor grafts ensures a high engraftment rate, good immune reconstitution, low incidence of significant aGvHD especially visceral GvHD and acceptable post-transplant morbidity in children with a range of PIDs even when used for second HSCT. CD3+TCR $\alpha\beta$ ⁺/CD19⁺ depleted HSCT from an HLA-haploidentical parent is a viable option for children with PIDs and can also be considered as a therapeutic modality for patients with refractory GvHD. Prevention and successful treatment of viral infections are the primary determinants of a healthy transplantation outcome. Although our study confirms short-term tolerability of reduced toxicity myeloablative conditioning regimens for PID patients, longer follow-up is needed to evaluate long-term effects for example on fertility.

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Table I: Demographics and transplantation characteristics of patients

No	Age (Years)	Sex	Diagnosis	Immediate Pre-transplant morbidity	Donor	Conditioning	GvHD Prophylaxis	Engraftment Day Post D0 (Neutrophil ⁺ /Platelet*)	GvHD (Grade)	Systemic Viral Infection Post D0	Latest Chimerism	Last Follow Up Day/ Outcome
1	1 yr	M	WAS (c.778-1G>A mutation in intron 8)	Disseminated CMV	Father	Bu (80mg/L/hr) /Flu/TT rATG [±]	CsA+ MMF	15/17	never	CMV	100%	1060/ Alive+ well
2	5 m	M	WAS [c.1480delp.(leu470*) hemizygote exon 11 of WAS gene]	Developmental delay and hypotonia(? cause), pulmonary hemorrhage	Mother	Treo/Flu/TT rATG/Ritux	CsA	11/9	never	none	100%	368/ Alive+ well
3	1.17 yr	M	WAS (c777+1 G>A splice site mutation in exon 7)	ALL in morphological remission/High risk MRD post induction. Prolonged cytopenias post chemo	Mother	Treo/Flu/TT rATG	CsA	15/18	never	CMV retinitis CMV CTLs	100%	1131/Alive and well
4	1.7 yr	M	WAS (c777+1 G>A splice site mutation in exon 7)	Autoimmune Neutropenia	Mother	Treo/Flu/TT rATG	None	21/10	Gr II skin	CMV CTLs	100%	935/alive and well
5	6 m	M	CyC SCID (IL2 RG gene c.294dup p.(Val992serf*s11) hemizygote)	Disseminated BCG	Father	None	CsA+ MMF	0/0	never	none	T-97% B-10% Myloid-6%	965/ Alive+ well

6	3 m	F	SCID (T-B+NK+)	None	Father	None	CsA+ MMF	0/0	never	none	T-94% B-0% Myeloid-0%	798/ Alive+ well
7.	1.7 yr	F	Artemis deficient SCID (homozygous c.103C>G exon 1 DCLRE1C) Refractory GvHD (11 months after cord SCT)	Refractory Skin GvHD (11 month after cord SCT), TPN dependent (Diarrhoea)	Mother	Treo/Flu/TT Alemtuzumab	Tac/CsA + MMF+ steroids	12/12	Skin (II)	Adeno	100%	1217/ Alive+ well Mild renal insufficiency (TMA injury)
8	5.3 yr	M	CyC SCID (c.854G>A in exon 6 of IL2Ryc) + GvHD (9 month post cord SCT)	Refractory Skin GvHD with joints involvement and marrow aplasia. ?Fungal pneumonia	Father	Treo/Flu Alemtuzumab	CsA+ MMF+ steroids	13/24	Skin (I)	HHV-6 Adeno	100%	634/ Alive+ well Joint Problems resolved
9	1.2 year	F	SCID- RAG2 (heterozygous variant C.457>C) (6 months post cord SCT)	Refractory Grade III skin, gut and Liver GvHD	Father	Treo/Flu/TT rATG/Rituximab	CsA+ MMF+ Steroids	15/08	Skin (I)	None	100%	155/ Alive and Well
10	5 m	F	SCID -RAG2 (c1247G>T in exon 2)	Disseminated CMV (pneumonitis/encephalitis)—T cell mediated pneumonitis? On ventilator	Father	Alemtuzumab	CsA	Early death	n/a		NA	9/ died due to ALI (T cell infiltration in biopsy)

11	10 m	M	Artemis deficient SCID	Pneumonia (cause?)	Mother	Treo/Flu/TT rATG/Ritux	CsA	15/10	never		T-100% B-92% M-55%	130/alive and well
12	8 m	M	Omenn syndrome (RAG1 SCID)	Adenoviremia, Glaucoma on D-2	Mother	Treo/Flu/TT rATG	CsA+ Steroids	10/13	never		NA	51/ Died because of ALI/PHTN
13	4 m	F	Omenn syndrome (IL7R compound heterozygous mutation)	Skin rash, Diarrhoea	Mother	Treo/Flu/TT rATG	CsA steroids	27/12	never	Adeno	T-100% B-34% Myloid-25%	530/ Alive+ well
14	1 yr	M	CID	Disseminated resistant CMV, Renal tubulopathy	Mother	Treo/Flu/TT	CsA+ MMF	11/0	Skin (III)	CMV^ (MDR) Maternal CTLs on D+7	100% till death	287/ Died (prolonged IS and Aspergillosis)
15	7.4 yr	M	DOCK 8 Deficiency (homozygous deletion of exon 8)	Disseminated adeno and CMV, warts	Mother	Treo/Flu/TT Alemtuzumab	CsA+ MMF	12/5	never	CMV gut disease	100%	1150/ Alive+ well, mild bronchiectasis for a year post HSCT (now resolved) warts started to fall after D+11
16	3.7 yr	M	CGD (p22 deficiency)	Disseminated BCG, granulomatous pneumonitis (nodules)	Father	Treo/Flu/TT Alemtuzumab	CsA+ MMF	14/10	Skin (I)	CMV Adeno^ HHV-6	100%	750/ Alive+ well
17	2.4 yr	M	CGD (X linked)	Enteropathy on PEG feed	Mother	Treo/Flu/TT rATG	CsA	15/0	Skin (II)	Adeno HHV-6^	100%	388/ Alive+ well

18	1.9 yr	M	CD40 Ligand Deficiency (C.540del.(Arg181 Glyfs*10) exon5)	None	Father	Treo/Flu/TT rATG	CsA	12/8	Skin (I)	CMV Adeno HHV-6	T-96% B-96% Myloid- 83%	698/ Alive+ well
19	2.6 yr	M	MHC-II Deficiency (RFXANK homozygous del- c.362A>Tp.D121V)	None	Mother	Treo/Flu/TT rATG	CsA	16/8	Skin (I)	Adeno (maternal CTLs on D47)	100%	413/ Alive+ well
20	1.8	F	MHC-II Deficiency (RFXP2c.362 mutation)	None	Father	Treo/Flu/TT rATG/ Rituximab	CsA	15/9	never	Enterovir us^ Meningo encephali tis- hydrocep halus with immune reconstit ution	100%	334/ Alive+ and doing well, Poor weight gain (Noro and enterovirus related enteropathy?)
21	4 m	F	HLH (MUNC13-4; c817c>tpR273x)	Disseminated CMV (CMV pneumonitis)	Father	Treo/Flu/TT rATG	None		n/a	CMV pneumon itis	NA	13/ died due to severe PHTN
22	2.7	F	HLH	Transaminitis	Father	Treo/Flu/TT rATG/ Rituximab	CsA+ MMF	18/10	never	None	100%	483/ Alive+ well
23	10.3	F	STAT3 GOF (exon22c.2144C>T)	Neutropenia, Autoimmune enteropathy Disseminated adeno	MMU D	Treo/Flu/TT rATG/ Rituximab	CsA+ steroids	20/7	never	HHV-6^	100%	475/ Alive+ well

24	2.7 yr	M	IPEX Syndrome	Failed MMUD cord transplant	Mother	Treo/Flu/TT rATG	None	18/0	Gr I skin		100%	365/alive and well
25	9.3 yr	M	SCN with MDS (ELANEC.688DEL G)	MDS,+ enteropathy	Mother	Treo/Flu/TT rATG	None	Primary Graft failure			NA	29-Primary graft failure
26	9.4 yr		Same patient	failed 1 st Transplant	MMU D	TBI(4Gy)/Flu/ TT/Cyclo(60) rATG [‡]	CsA+ MMF	10/0	Gr II skin	CMV Pneumon itis	100%	850/Alive and well

Abbreviations: ALI- Acute Lung Injury, Bu-Busulfan, CGD- Chronic Granulomatous Disease, CID- Combined Immune Deficiency, CMV- Cytomegalovirus, CTLs- Cytotoxic T Lymphocytes, C γ C- Common γ Chain, DOCK 8- Deducator of Cytokines 8, Flu- Fludarabine, GvHD- Graft versus Host Disease, HLH- Hemophagocytic Lymphohistiocytosis, , HHV-6-Human Herpes Virus type-6, HR- high risk, IS- Immunosuppression, IPEX syndrome- Immunodysregulation Polyendocrinopathy Enteropathy X-linked syndrome, mMUD- Mismatched unrelated donor, MRD- Minimal residual disease, PHTN- Pulmonary Hypertension, RAG- Recombinase-activating gene, rATG-rabbit ATG, SCID- Severe Combined Immune Deficiency, SCN- Severe congenital neutropenia, SCT- Stem cell transplant, STAT3 GOF- STAT 3 Gain of Function, TBI- Total body irradiation, TMA- Thrombotic Microangiopathy, Treo- Treosulfan, TT-Thiotepa, WAS- Wiskott Aldrich Syndrome

† Neutrophil engraftment: ANC >0.5x10⁹/L for 3 consecutive days

*Platelet engraftment: Platelet count >20x10⁹/L for 7 consecutive days without transfusion support (for WAS patients the cut off was >50x10⁹/L for 3 days without transfusion support)

^ No specific antiviral treatment given

‡ATG dose 15mg/kg except patient 26 where lower dose used (6mg/kg)

Table II: Comparison of current study with previously published evidence using same method of TCD in children with PIDs

	Bertaina et al. ¹⁶	Balashow et al. ¹⁵	Current study
No of patients (n)	23 (PID=11)	37	25
Median Age (years)	3.3 (0.4-12)	2.6 (0.2-17)	1.75 (0.2-10.3)
Donors (n)	Haplo (23)	MUD (27) Haplo (10)	MMUD (2) Haplo (24)
Serotherapy	rATG 12mg/kg + Ritux 200 mg/m2 (all)	rATG 10mg/kg (20) rATG + Ritux 100mg/m2 (14) Alemtuzumab (2)	rATG 15mg/kg (11) rATG+ Ritux 200mg/m2 (6) Alemtuzumab (5) None (3)
Median cell dose (range)			
CD34 cells x10 ⁶ /kg BW	15.8 (10.2-40)	11.7 (5.9-21.3)	17.8x10 ⁶ (4.7-50.9)
TCR αβ cells x10 ⁴ /kg BW	4 (1-9.5)	1.6 (0.08-36.8)	3.3 (0.075-9.5)
B cells x10 ⁵ /kg BW	0.4 (0.05-1.5)	NA	4 (0.038-11)
NK cells x10 ⁷ /kg	3.82 (1.57-17.6)	NA	5.15(0.13-15)
GvHD prophylaxis	None	Tac+MTX (34) Tacro + MMF (2) CsA +MTX (1)	CsA or Tac +MMF (11) CsA (11) None (4)
Neutrophil engraftment (days)	13 (10-20)	16 (11-28)	15* (10-27)
CI (%)Primary/Secondary Graft failure	8.7/8.7	5.4/21.6	4.2/0
CI (%) aGvHD	Gr I/II- 13.1 Gr III/IV-0	Gr II-21.5 Gr III/IV-2.8	Gr I/II-44.1 (95% CI 27.7-70.2) Gr III-IV-4.3 (95% CI 0.6-29.6)
cGvHD	None	1 patient	None
CI (%) Systemic virus infection/reactivations	38% for CMV and adeno	46% for CMV	35.0 ±9.4% for CMV, 33.2±9.7% for Adeno, 26.3±10.4% for HHV-6

TRM at one year (%)	9.3	3.3	16.4
Median follow up in months (range)	18 (5-40)	13 (4-29)	20.8 (5-40.0)
EFS/OS (%)	74/91.1	67.7/96.5	80.4/83.9

Abbreviations: aGvHD- acute graft versus host disease, BW- body weight, CI- cumulative incidence, CMV- Cytomegalovirus, EFS- event free survival, Haplo- Haploidentical, Ritux- Rituximab, PBSC- peripheral blood stem cells, Tac- Tacrolimus, TRM- transplant related mortality, OS- Overall survival

*unconditioned data excluded

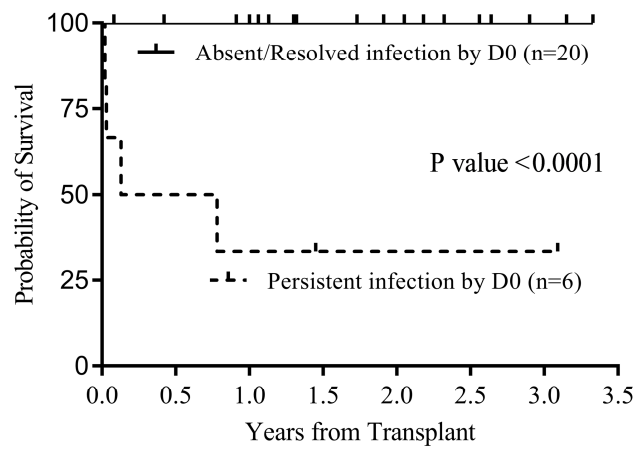
Figure 1: Survival according to CMV or Adenoviral infection status on D0

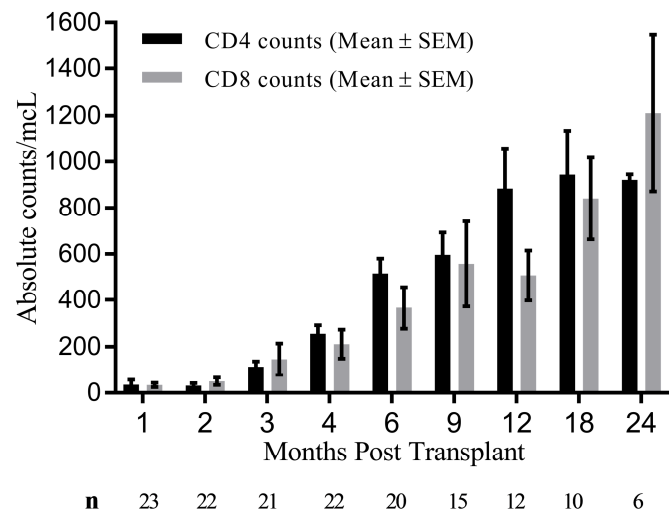
Figure 2: CD4 and CD8 cell reconstitution post-transplant

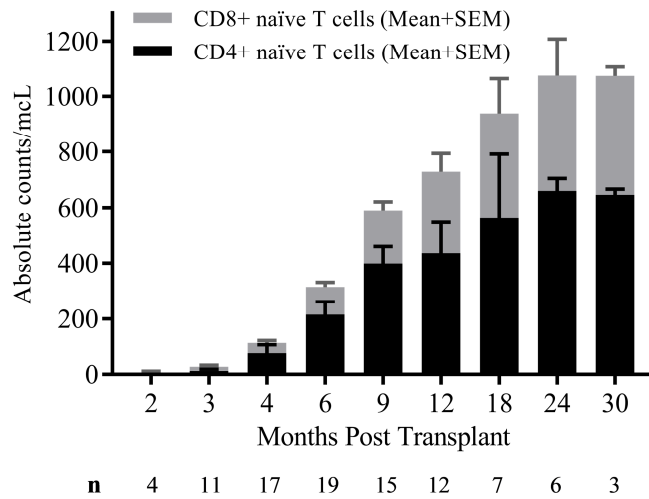
Figure 3: naïve T-cell recovery

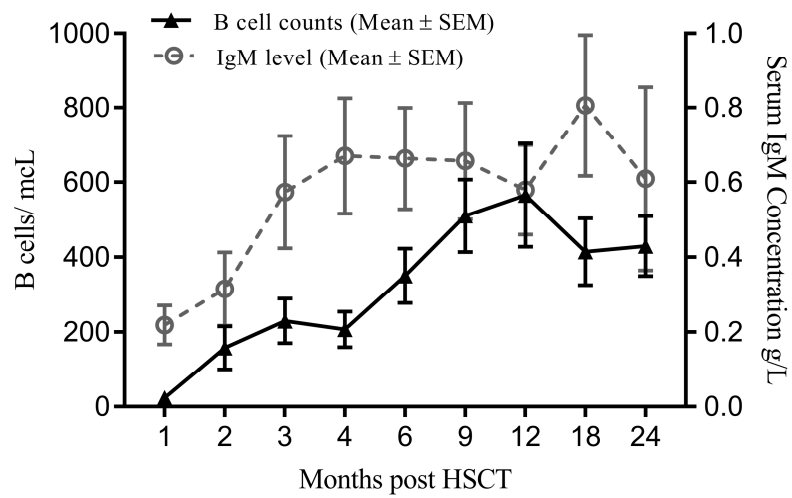
Figure 4: B-cell* and IgM recovery post-transplant (*B+ SCIDs excluded)

Figure 5: $\alpha\beta$ and $\gamma\delta$ T-cell reconstitution post-HSCT









n (B cell) 20 20 19 19 18 14 10 7 6

n (IgM) 10 10 11 13 13 10 10 8 4

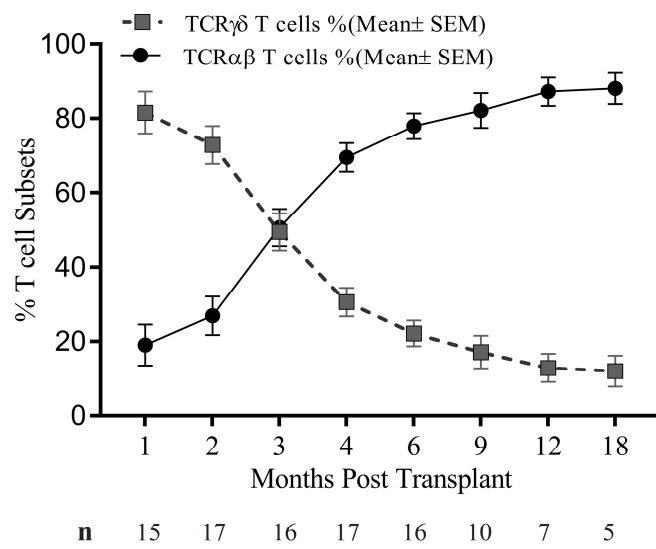


Table S1: Comparison of current study with previously published evidence using different method of TCD in children with PIDs

	Pai S et al ³³ .	Buckley et al ³² .	Current Study
Number of mismatched transplants	138 (SCID)	77 (SCID)	26 (PID)
TCD method	Soybean agglutination and E-rosette TCD (51%) CD34 selection (36%)	Soybean agglutination and E-rosette TCD of Marrow	TCR $\alpha\beta$ CD3+ cells and CD19+ cells depletion of PBSC
Conditioning	MA (18%), RIC (7%) Immune suppression (12%) None (63%)	None except 2 patients who had previous transplant	MA (88.5%) None (11.5%)
GvHD prophylaxis	4%	None except 2 who received conditioning	84.6%
OS	66% with conditioning 79% without conditioning	83.9%	83.9%
CI (%) aGvHD Gr III-IV	10	10.4 (involving skin, gut)	4.3 (No visceral GvHD)
CI (%) Chronic GvHD	16	NA	0
CI (%) Graft Failure	24	19.5	4.2
B cell chimerism	39% (13/36)	NA	81.5% (18/22)
CD4 T cells >500cells/ μ L at >2 year follow up	30%	NA	83.3% (5/6)
Off immunoglobulin replacement at 2-5 years	37%	49.5%	82.3%

Abbreviations: aGvHD- acute graft versus host disease, CI- cumulative incidence, HSCT- Hematopoietic stem cell transplantation, MA- Myeloablative, PBSC- peripheral blood stem cells, PID- primary immune deficiency, RIC- reduced intensity conditioning, SCID- severe combined immune deficiency, TCD- T cell depletion, OS- Overall survival